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D-Lactic Acid Measurements in the Diagnosis of Bacterial Infections

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Body fluids suspected of bacterial infection were cultured and examined for the presence of D-lactic acid, a specific bacterial metabolite. We examined 206 patients and 264 specimens. D-Lactic acid was found in concentrations of greater than or equal to 0.15 mM in 11 of 11 infected and 6 of 40 noninfected ascitic fluids, 6 of 6 infected and 4 of 33 noninfected pleural fluids, 4 of 4 infected and 0 of 13 noninfected synovial fluids, and 26 of 27 infected and 2 of 130 noninfected cerebrospinal fluids. The overall sensitivity was 79.7%, and the specificity was 99.5% when the D-lactic acid concentration was at least 0.15 mM. The most important clinical utility of the D-lactic acid measurement appears to be for patients with bacterial infection in various body compartments and in patients who have already received antimicrobial therapy. An elevation in D-lactic acid may indicate the presence of bacterial infection even when cultures are negative.

The presence of bacteria in body fluids has been detected by direct microscopic examinations of the fluid (1). Many attempts to improve upon this method of rapid diagnosis have met with only varied degrees of success. These techniques include antigen detection (7, 13, 20), L-lactic acid measurements (3), pH measurements (16), *Limulus* lysate assays (17), and the detection of bacterial metabolites in body fluids (2, 8, 10, 15, 21). Bacterial antigen detection has been available for only a limited number of bacterial species (7, 20), and the other methods have not gained popularity or attained wide acceptance either because of a lack of sensitivity and specificity or because the procedure is too difficult or cumbersome for routine use in a clinical laboratory.

D-Lactic acid is a specific metabolite of lower forms of life (4, 5) and, when found in normally sterile body fluids, indicates bacterial invasion or absorption from a site containing a high density of bacteria (18). This metabolite has been implicated in cattle foundering (6) and in humans with acidosis due to short bowel syndrome (14, 19). In both instances, the high blood D-lactic acid concentrations are postulated to result from increased production of the metabolite in the gastrointestinal tract by the bacterial flora with subsequent absorption into the blood.

The determination of D-lactic acid is not difficult and is comparable to that for measuring L-lactic acid, an already established clinical laboratory test (22). In this study, the utility of D-lactic acid measurements in distinguishing infected from noninfected body fluids was assessed.

MATERIALS AND METHODS

Patient population. Three metropolitan hospitals contributed clinical samples for examination: East Orange Veterans Administration Medical Center, East Orange, N.J., Children's Hospital National Medical Center, Washington, D.C., and the Veterans Administration Medical Center, Bay Pines, Fla. Specimens were sent to the respective pathology or laboratory medicine department and processed according to the needs of the primary care physician team. In all cases, bacterial cultures were requested. The remainder of the samples was immediately placed in 2 volumes of 7% (vol/vol) perchloric acid and well mixed. Samples were mailed to the test site, the East Orange Veterans Administration Medical Center. Clinical information such as source of the sample, site of infection identified, prior antibiotic treatment, and the bacterial organisms isolated from the fluid were recorded as part of the study. This study of laboratory specimens was exempt from any requirement for informed human consent.

D-Lactic acid assay. Each sample that was previously diluted in 2 volumes of 7% perchloric acid was mixed and centrifuged at 1,500 × *g* for 20 min. The supernatant fluids were assayed by the procedure of Smith et al. (18). D-Lactate dehydrogenase, glycine buffer (pH 9.2), and NAD were purchased from Sigma Chemical Co., St. Louis, Mo., and were added to all test samples. Individual sample blanks contained the sample and water. Simultaneous samples with known amounts of D-lactic acid (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) were also tested to generate a standard curve. All tubes were incubated in a 35°C water bath for 1 h, and the A_{340} was determined with a Gilford UV-Vis model 300N spectrophotometer (Gilford Systems, Oberlin, Ohio). The sample D-lactic acid concentrations were determined by interpolation of points generated from the standard curve.

RESULTS

Fluid sample characteristics. From the three medical centers, a total of 206 patients had 264 body fluid samples analyzed. These fluids were from such varied sites as the pleura (n = 39), synovia (n = 17), peritoneal cavity (n = 51), and the cerebrospinal fluid (CSF) (n = 157). Ten were repeat specimens performed at 48 h into antimicrobial therapy and after the initial fluid showed an elevated D-lactic acid level. Five repeat samples were CSF, two were pleural, two were synovial, and one was ascitic. All repeat samples (48 h into appropriate therapy) showed persistence of elevated D-lactic acid levels. One of the pleural fluids had a D-lactic acid level that decreased from 0.17 to 0.11 mM, whereas the other had

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TABLE 1. Summary of bacterial species involved

Fluid type	No. of specimens	Bacterial species
Ascitic	3	*Streptococcus faecalis*
	3	*Staphylococcus aureus*
	1	*Streptococcus* sp. group B
	1	*Streptococcus* sp. group G
	1	*Escherichia coli*
	1	*Klebsiella pneumoniae*
	1	*Campylobacter intestinalis*
Pleural	2	*Staphylococcus aureus*
	1	*Staphylococcus epidermidis*
	1	*Streptococcus* sp. group A
	1	*Streptococcus pneumoniae*
	1	*Escherichia coli*
Synovial	1	*Staphylococcus aureus*
	1	*Streptococcus* sp. group G
	1	*Escherichia coli*
	1	*Enterobacter cloacae*
CSF	6	*Staphylococcus epidermidis*
	6	*Streptococcus* sp. group B
	4	*Streptococcus pneumoniae*
	2	*Staphylococcus aureus*
	2	*Hemophilus influenzae*
	2	*Escherichia coli*
	1	*Neisseria meningitidis*
	1	*Enterobacter agglomerans*
	1	*Enterobacter cloacae*
	1	*Serratia marcescens*
	1	*Citrobacter diversus*

an increase from 0.5 to 0.9 mM. The synovial fluid levels decreased from 0.19 to 0.14 mM and from 0.17 to 0.12 mM, respectively. The patient with repeat ascitic fluid samples showed a decrease in D-lactic acid from 0.42 to 0.34 mM. Organisms involved in the CSF and in the other body fluid infections are listed in Table 1. The chemical analyses of body fluids (glucose, specific gravity, and L-lactate dehydrogenase) rarely provided specific information on infection and were not included in this analysis. Gram stain examinations were performed on all samples and indicated the presence of bacteria whenever the cultures were positive except in three samples of CSF.

D-Lactic acid levels. The results of D-lactic acid measurements are shown in Fig. 1 and 2 for the various fluid samples analyzed. Repeat specimens, a total of 10, are not included in the figures or calculations. Figure 1 shows the distribution of D-lactic acid concentrations in infected and noninfected fluids. There were no infected fluids with a D-lactic acid concentration of less than 0.1 mM and no noninfected fluids with concentrations of greater than 0.2 mM. However, there was an overlapping range between 0.1 and 0.2 mM for infected and noninfected fluids. The distribution of D-lactic acid concentrations in CSF is shown in Fig. 2. The D-lactic acid levels for noninfected fluids were less than or equal to 0.11 mM except for two samples shown on the left side of the figure. D-Lactic concentrations in bacterial meningitis were greater than 0.11 mM except for one sample.

When the criterion of 0.15 mM was chosen to represent a positive test, the overall sensitivity of the assay was 79.7% with a specificity of 99.5%. The individual sensitivities and specificities are shown in Table 2. The CSF group had the greatest number of samples analyzed (157); for this source the sensitivity and specificity were 92.3 and 99.2%, respectively. The sensitivities for ascitic and pleural fluids were

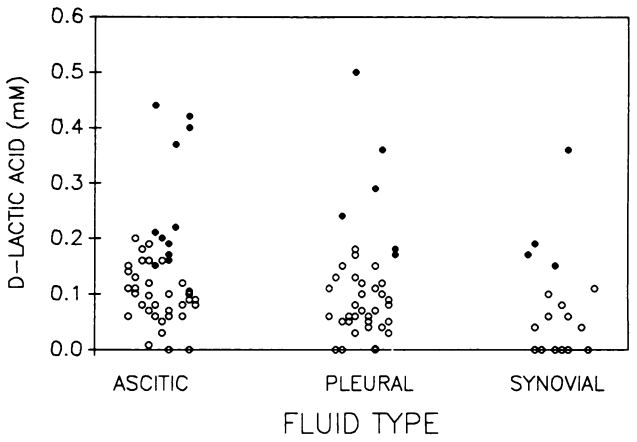

FIG. 1. D-Lactic acid levels in normally sterile body fluids. Symbols: ○, fluids from noninfected patients; ●, culture-positive fluids from patients who were clinically infected.

lower with 64.7 and 60.0%, respectively. Although the sensitivity of the tests for synovial fluids was high by comparison, this value may reflect the small sample size examined.

DISCUSSION

The utility of D-lactic acid measurements for the diagnosis of bacterial infections was examined for normally sterile body fluids. At most sites of infection there is a reduced redox potential, which induces the bacteria to switch to anaerobic or microaerophilic metabolism, e.g., to produce lactic acid rather than CO_2 as the major end product of metabolism (9). Although all organisms may not produce significant quantities of D-lactic acid (e.g., streptococci), previous work (18) indicates that D-lactic acid is accumulated during growth of organisms in vitro and during experimental infections. From the variety of bacterial species involved and the variety of sources of body fluids studied, it appears that D-lactic acid does satisfy the need of being specifically present in infected fluids regardless of the species involved. The presence of very small amounts of D-lactic acid found in all types of body fluids complicates the interpretation of this measurement. The low levels of D-

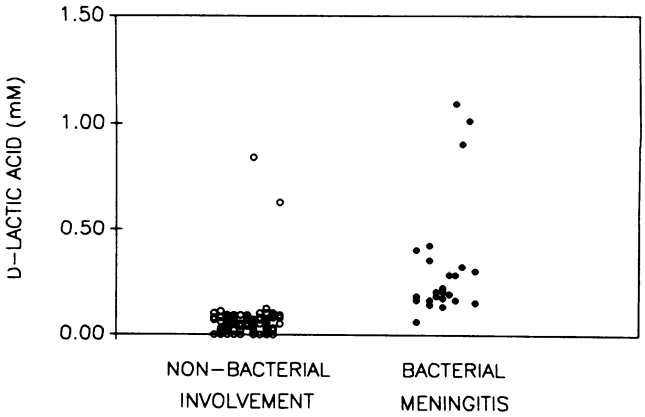

FIG. 2. D-Lactic acid levels in CSF. Symbols: ○, samples from patients with diseases other than bacterial meningitis; ●, samples from patients with bacterial meningitis.

TABLE 2. Summary of D-lactic acid results[a]

Source (*n*)	No. of positive cultures			No. of negative cultures			% Sensitivity[b]	% Specificity[c]
	Total	TP with elevated D-lactate	FN with normal D-lactate	Total	FP with elevated D-lactate	TN with normal D-lactate		
Ascitic fluid (51)	11	11	0	40	6	34	64.7	100
Pleural fluid (39)	6	6	0	33	4	29	60.0	100
Synovial fluid (17)	4	4	0	13	0	13	100	100
CSF (157)	27	26	1	130	2	128	92.3	99.2
Overall							79.7	99.5

[a] TP, True positives; FN, false-negatives; FP, false-positives; TN, true negatives. The cutoff value for normal D-lactate (lactic acid) used was 0.15 mM.
[b] Sensitivity = TP/(TP + FN) × 100.
[c] Specificity = TN/(TN + FP) × 100.

lactic acid are postulated to result from the absorption of D-lactic acid produced in the intestinal tract.

In this study of normally sterile body fluids (ascitic, pleural, synovial, and CSF), the overall sensitivity of finding D-lactic acid in concentrations of greater than or equal to 0.15 mM was 79.7% with a corresponding specificity of 99.5%. When only CSF results were examined, the sensitivity increased to 92.3%, a value which exceeds sensitivity reported for the Gram stain when looking at composite data for CSF (13). The sensitivity of the Gram stain for CSF varies greatly, with averages from 45 to 80% depending upon the pathogen involved (12, 13). In more quantitative studies, La Scolea and Dryja (11) reported that the sensitivity of the Gram stain varies between 25 and 97% depending upon the concentration of bacteria in the CSF. In our study the sensitivity of finding a D-lactic concentration of at least 0.15 mM was comparable to or greater than that of the Gram-stained smear.

To date, no rapid test has emerged that can exceed the sensitivity of the Gram stain for detecting a wide range of bacterial pathogens when greater than 10^5 CFU/ml are present (11). The Gram stain has the advantage that when positive in a normally sterile body fluid, therapy can generally be directed toward either Gram-positive or Gram-negative organisms or both. Unfortunately the Gram stain has inherent problems such as the presence of high protein or the fact that an excessive amount of exudate may make interpretation difficult. In addition, when bacterial counts are low, the smear results may be negative. However the data presented here indicate that the presence of an elevated body fluid D-lactic acid level can identify those body fluids in which bacterial metabolism has occurred, although it cannot specifically identifying the genus or species of bacteria involved. When a D-lactic acid measurement indicates the presence of bacteria in a body fluid specimen, the clinician may proceed with greater confidence in administering therapy, although the presumptive identification is lacking.

D-Lactic acid measurement is of particular utility as a supportive test to the Gram stain when the specimen is from an infected patient who has already received antibiotic therapy. In this scenario the morphology of the bacteria may be so altered by antibiotic action that its recognition may be difficult or impossible, and the culture of the sample may be negative. In this situation the presence of a D-lactic acid level of greater than 0.15 mM may be of critical importance in helping to consolidate the decision of whether to treat or continue treatment for a bacterial infection at that site.

The D-lactic acid assay is simple to perform and requires only a spectrophotometer with 340 nm capability and should therefore be easily adaptable for use in most clinical laboratories. In view of the discriminatory ability of this test to identify fluids containing a wide variety of bacterial pathogens, the D-lactic acid assay should be considered for use whenever rapid diagnosis is critical to the management of patients in whom bacterial infection is suspected and in whom a Gram-stained smear has been negative.

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